5   METHOD FOR TREATING ONCOLOGICAL, VIRULENT
AND SOMATIC DISEASES, METHOD FOR CONTROLLING
TREATMENT EFFICIENCY, PHARMACEUTICAL AGENTS AND
COMPOSITION FOR CARRYING OUT SAID TREATMENT

10   Technical field

The invention relates to medicine and veterinary science and discloses a novel method for treating oncological, virulent and somatic diseases when the main target for therapeutic action is embodied in the

15   form of DNA which freely circulates in blood plasma (and other liquid media) and originates from tumoral and mutant cells or cells infected by bacteria, fungi or protozoan and from different microorganisms which reside in the organism thereof. Said invention also relates to novel pharmaceutical compositions and to the use thereof for treating

20   oncological diseases and infectious states provoked by bacteria, fungi and protozoa and non-infectious somatic diseases and states produced by accumulation of somatic mutations in cells of organism. Medicinal and immunological compositions, sorption and physico-chemical engineering and the method for using them in order to treat malignant

25   tumors and to prevent their relapses as well as for treatment of infections, atherosclerosis, diabetes and slowing the aging process. Proposed method is based on completely new principle of therapeutic

action, provide strong antitumor and antimicrobial efficacy and could be used in therapy of oncological diseases, different infections and non-infectious diseases.

5   Background art

Populations of cancer cells, developing in patient's organism possess extremely high level of genetic plasticity that is much higher than that in normal cells. Genetic plasticity within cancer cells

10   populations allows them to generate in course of disease the phenotypes which are resistant to immune and morphogenic control and which has the capacity for invasion and metastasis and are resistant to antitumor therapies. It is known, that selection and clonal expansion of tumor cells comprise the basis of biological and clinical tumor progression.

15   According to that knowledge, the modern strategy of anticancer therapy is based on principles of tumor cell clones destruction in patient organism through chemotherapy, radiotherapy, immunotherapy, surgical elimination and by use of their combinations. Fundamental particularity of all these methods is that their sole therapeutic target is a tumor cell.

20   The long term experience from clinical application of such therapy has shown that due to high genetic placticity the most malignant cells become insensitive to the specific therapy used before malignant neoplasm could be completely destroyed by said therapy.

There is a great medical need for new antitumor drugs less toxic

25   than existing ones or can potentiate thre efficacy of existing methods. As well it is a need for new antitumor drugs that can decrease toxicity of currently available methods whithout decreasing of their efficacy.

The circulation of DNA molecules in blood plasma of cancer patients and healthy people was known from some publications (P.Anker et al., Clinica Chimica Acta,v.313, 2001, pp.143-146; Fedorov N.A. et.al., Bull.Exp.Biol.Med.,v102,1986, pp281-283. Patent (US 5952170)

5   disclouse the determination of DNA content in blood plasma for diagnostic and prognostic purposes in course of cancer disease. Patents (US 6465177 and US 6156504) disclouse the use of blood plasma DNA for mutation's determination in oncogenes and microsatellite gene regions, for genetic instability studying in tumors and the use of these

10   results for diagnostic, monitoring and prognostic purposes in the course of disease.

Sugihara S. et al. (1990, 1993) studied ⊡chemotrypsine and desoxiribonuclease I (DNase I) enzymes influence on autologic and heterologic adhesion of tumor cells during their metastasing. They have

15   shown that systemic introduction of DNase I could slow metastases development. The effect they find was therapeutically insufficient. Authors make conclusion that DNase I can be used together with surgical elimination of tumor for prevention of tumor cells' hematogenic dissemination. Authors meant the influence of DNase I on tumor cells'

20   cytoplasmatic membrane but not on destruction of free circulating DNA. Accordingly, the regime and doses that were used could not cause the decrease of circulating DNA level.

Torchilin V.P. (2001), Patent (US 5,780,033), disclouse the therapeutic use of autoantibodies that are able to bind with cytoplasmic

25   and nuclear membranes of tumor cells and with protein-DNA complex coming from dead tumor cells. It is clear from the text of application that the disclousure relates solely to antibodies against protein antigenic

determinants. In our case anti-DNA antibodies and anti-DNA abzymes are used. Moreover, therapy discloused by authors was trended against phagocytosis of nucleosomes on the surface of tumor cells and that exclude the possibility of therapeutic regimes sufficient for binding and removal of circulating blood plasma DNA.

There is practically no data concerning circulation of bacterial DNA in blood. In human organism all microorganisms exist in biofilm communities (Davey M.E. Otoole G.A. 2000. Microbial biofilms: from ecology to Molecular ganetics. Microbiol. Mol.Genet. 64:847-867). Biofilms are formed by bacterial cells, associated with extracellular matrix (Tez V.V. 1999. Formation and structure of mixed bacterial communities. APMIS, 107:645-654). In biofilm matrix we have found extracellular DNA that penetrates from living cells. Our data also indicates that bacterial DNA present in blood plasma of infected human and its quantities and composition could change subject to development of certain infections. It is also known that DNA can get into the outer environment as a result of cells death, for example in the focus of inflammation. DNA as polymer increases biofluids viscosity and that negatively influence the course of the disease, preventing elimination of toxins, pathogens and necrotic debris. It is known that-DNase (Gentech-Roch) "Pulmozyme" is used for Cystic Fibrosis treatment by inhalation. The therapeutic action is based on dilution of broncho-alveolar secret, and does not relate to breach of genetic information traffic.

There is no human or animal data, regarding the genetic composition of DNA, circulating in blood plasma. There is no data regarding blood plasma DNA analysis avoiding PCR (Polymerase Chain Reaction). The use of PCR can considerably distort the representation of

DNA composition because of amplification primer's specificity. So far , the genetic analysis of plasma DNA in cancer patients were done by PSR method or blot hybridization and was directed to studying of genome changes in certain specific genome areas (for example in microsatellites and specific genes).(Sanchez-Cespedes M., et al., Ann Oncol,1998,v9(1),pp113-116; Sozzi G., et al., Clin Can Res, 1999, v5(10),pp2689-2692; Chen X.Q., et al., Nat Med,1996,v2(9),pp1033-1035).

Thus, there is no data available about genetic repertoire of DNA which circulates in blood plasma of patients with oncopathology, infections, somatic pathology and healthy people. , about the biological role of of blood plasma circulating DNA and about possible therapeutic effect of blood plasma circulating DNA destruction or inactivation in these diseases treatment.

Disclosure of the invention

As a result of our work upon the invention we have unexpectedly found that blood plasma circulating DNA from oncological patients contain unique quantitative and qualitative repertoire of genes and regulatory genetic elements, which is strongly different from that of human genome. Circulating blood plasma DNA of oncological patients for the most part contains unique human genes, including genes that are associated with maintenance and formation of malignancy.

It was shown that circulating blood plasma DNA of patients with oncopathology participates in intercellular transport of genetic information within tumor cells' population in patient organism. Current

invention disclosure methods of free blood plasma circulating DNA destruction and inactivation which lead to suppression of tumor development in host organism. The invention also disclosure new method for identification of genomic structures which are involved in

5   tumor progression and human genome operation. This aspect of invention is connected with DNA samples isolation from blood plasma of healthy people and patients with oncological diseases, cloning and sequencing them.

It was found out that DNA from biofilm matrix secreted by

10   different bacteria enters into the blood and biological fluids of human and animals. It was stated that the presence of extracellular DNA is one of conditions necessary for microbial infection development.

Invention includes destruction or inactivation of circulating microbial DNA as method for treatment and prophylaxis of diseases

15   caused by them.

It was also found out that DNA circulating in blood of healthy people play role in somatic mosaicism's development. Binding, destruction or inactivation of that DNA could suppress the somatic mosaicism development. Binding, destruction or inactivation of

20   circulating blood plasma DNA could be of therapeutic value in treatment of diseases which progression is linked with somatic mosaicism development.

One aspect of invention discloses the pharmaceutical compositions and non pharmaceutical methods of free circulating DNA destruction or

25   inactivation in blood plasma of patients with oncological or infectious diseases.

Another invention aspect discloses treatment method of patients' with oncopathology, infections, somatic diseases and method of extension of life time, which are based on administration of pharmaceutical compounds or application of non pharmaceutical

5   methods providing the destruction or inactivation to free circulating blood plasma DNA.

Another aspect of invention provide the way of treatment efficacy control, when said treatment is directed to destruction or inactivation of free circulating blood plasma DNA, then the monitoring of free

10   circulating blood plasma DNA and determination of tumor-specific genetic markers or genetic microbial markers presence in blood plasma could be used for treatment efficacy control.

Methods of patients' treatment with oncopathology and infectious diseases based on pharmaceutical compounds administration or use of

15   non pharmaceutical methods that lead to destruction or inactivation of free circulating blood plasma DNA, when such treatment is combined with use of antitumor or antimicrobial therapy were disclosed.

Genetic placticity of tumor cells , which allows them to accumulate and maintain signs that comprise "malignant phenotype",

20   reveals itself on genetic and chromosomal levels by loss, acquiring or changing of DNA consequences, starting from single nucleoside up to whole chromosomes (Loeb K.R. et.al., Carcinogenesis, v21,2000,pp.379-385).

Source of such variability is thought to be a special *modus*

25   *operandy* of tumor cells' genetic apparatus. It is characterized by increased frequency of spontaneous mutations while activity of reparative system is decreased and control system of genetic homeostasis

is switched off (Schmutte C., et al., Anticancer Res., 1999, v19, pp.4665-4696).

It is thought that "mutator" phenotype of tumor cells (Loeb L.A.,Cancer Research,2001, v61,pp.3230-3239), dynamic heterogeneity within tumor cell clones (Heppner G.H. et al., International Review of Cytology, 1998, v177, pp.1-56) and numerous rounds of selection in the course of progression (Cahill D.P. et al., Trends in Cell Biology, v9, pp.M57-M60 ; Rubin H., Adv Cancer Res, 2001,v83,pp.159-207; P.Nowell, Seminars in Cancer Biology, v 12, 2002, pp.261-266) lead to selection and following expansion of the most malignant tumor clones. According to this knowledge the modern methods of malignant neoplasm's treatment are based on principle of tumor cells destruction in patient organism.

In the process of investigation we have unexpectedly found that accumulation of genetic changes that are essential for "malignant phenotype" formation in clinically advanced tumor is a result of cooperative interclonal interaction inside population of tumor cells in patients' organism, based on horizontal transfer of genetic information

Free circulating DNA in blood plasma of oncological patients is a messenger of such cooperative relations. It realizes intrapopulational interclonal transport of genes, which are responsible for "malignant phenotype" formation.

Destruction or inactivation of free blood plasma circulating DNA will lead to inability of tumor cells to maintain necessary level of genetic variability in organism and loss ability to maintain "malignant phenotype" (growth, metastasing, insensitivity to therapy). Such

therapeutic intervention has its own therapeutic value as well as it increases efficacy of traditional therapeutic methods.

Brief description of the drawings

P-glycoprotein expression in murine tumors

Fig.1 Results of immunochemical staining of mice tumor's histological slices after administration of 5 day course of Doxorubicin therapy (2 mg/kg every day intravenous) and DNase I (0,5 mg/kg four times a day during 5 days).

A – Doxorubicin + DNase

B – Doxorubicin

Preferred embodiment

Isolation of free circulating DNA from blood plasma.

Fresh blood plasma (not more than 3-4 hours after isolation) with anticoagulant (sodium citrate) addition was centrifuged on Ficoll-PlaquePlus (Amersham-Pharmacia) step at 1500g for 20 minutes at room temperature. Plasma (1/2 of all amount) was neatly isolated avoiding touching cells sediment on ficoll step and was centrifuged at 10 000 g for 30 minutes to eliminate cells and debris. Supernatant was taken away not touching sediment and up to 1% of sarcosile, up to 50 mM of tris HCl pH 7,6, up to 20 mM EDTA, up to 400 mM NaCl and equal volume of phenol – chloroform mixture 1:1 were added. Received emulsion was incubated at 65^0C for 2 hours than phenol-chloroform was separated by centrifuging at 5000g during 20 minutes at room temperature.

Deproteinization by phenol-chloroform method was identically repeated for three times after what water phase was processed by chloroform and after it by diethyl ether.

Organic solvents' separation was done by centrifuging at 5000g during 15 minutes. Equal part of isopropanol was added to water phase and incubated during night at 0^0C.

After sedimentation nucleic acids were separated by centrifuging at 10 000g during 30 minutes. Sediment of nucleic acids was dissolved in buffer that consisted of 10 mM tris – HCl, pH 7,6, 5 mM EDTA and was inflicted on step made from chlorine cesium gradient (1M, 2.5M 5.7) in centrifuge test tube for SW60Ti rotor. DNA volume was 2 ml, volume of each CsCl step was 1 ml. Ultracentrifuging was done in L80-80 (Beckman) centrifuge for 3 hours at 250000g. DNA was isolated according to fractions from the step's surface 5.7M. Fractions were dialized during12 hours. mM tris-HCl, pH 7,6, 1mM EDTA at 4^0C will be added. DNA presence in fractions was defined by agarose electrophoresis with DNA visualization by ethidium bromide. DNA amount was spectrophotometrically estimated (Beckman DU 470) in cuvette with volume 100 mkl, using 220-320 nm spectrum. Average runout of DNA was 10-20ng according to 1 ml of plasma.

Cloning and sequencing of blood plasma DNA.

We have developed new method of DNA isolation and cloning from blood plasma, that allows to construct not amplified plasmide library of such DNA with representativeness up to million clones with average size 300-500 base pair isolated from 50 ml of blood, even taking into account significant amount of elevated liposaccharides level and non identified mixtures that troubled purification of nucleic acids. So

representative analysis can be done with less amount of plasma pattern – 10-20 ml depending on pathological contaminates' presence.

Isolated according to above-mentioned method DNA was deproteinized with the use of proteinase K (Sigma) at 65^0C for tightly-bound proteins elimination. After deproteinization DNA was processed by phenol-chloroform at 65^0C and sedimented by 2,5 volumes of ethanol during night. After it DNA was processed by EcoRI restrictase during 3 hours or by Pfu polymerase (Stratagene) at the presence of 300 mkM of all desoxynucleothydethreephosphates for "sticky" edges elimination. Completed DNA was phosphorylated by polynucleotide kinase T4 (30U, 2 hours). Received samples/preparations were ligated in Bluescript (Stratagene), plasmid digested by EcoR1 or PvuII accordingly and dephosphorylated by alkaline phosphatase CIP (Fermentas) during 1 hour. 1 mkg of vector and 0,1-0,5 mkg of serum DNA were usually used for ligation. Ligation was done by Rapid Ligation Kit (Roche) use for 10 hours at 16^0C. Volume of ligase mixture was 50 mkl. Ligated library was transformed into DH12S (Life Technologies) cells with electroporator (BioRad) use. For transformation of one library 12-20 electroporation cuvettes were used. Dilutions of the library at concentrations 10^{-4}, 10^{-5} and 10^{-6} were plated for control on dishes with 1,5% agar and LB media, supplemented with 100mkg\ml ampicillin. In both cases library's representativeness was approximately $2-3 \times 10^6$ clones.

Theoretically set of DNA sequences that circulate in plasma should correspond to set of genome's DNA sequences. Usually cells apoptosis is accompanied by quantitative and nonspecific DNA degradation before its exit out of the cell, so the most wide spread DNA

in plasma should be repetitive elements of genome in proportion that correspond to nonspecific degradation of DNA.

Such elements are L1 repeats, satellite DNA, Alu, MER, MIR, THE repeats and some others. Quantity of unique sequences should be small in accordance to their small percent in human genome they may be not detected in cloning DNA without PCR.

Blood plasma DNA library of oncological patient with clinically advanced tumor stage.

We have constructed blood plasma DNA library of patient with diagnosed advanced stage mesothelioma. Representativeness of library was $8,5 \times 10^5$ clones, that is a good result, taking into account rather small amount of DNA (5 □g) received after purification from non character for healthy donors liposaccharides that were in extremely high concentrations at plasma of patient.

We have got the unexpected results after analysis of 96 clones with length from 300 up to 1000 base pairs. (It is necessary to mention that only one clone was not identified as human DNA. For all others correspondent information from HumanGenBank that identifies DNA of these clones as human DNA was received.) As mentioned above, according to data from literature it is logically to assume that there will be a lot of higly repeatitive elements in DNA samples.

But at least 55 out of 96 clones presented unique sequences of human DNA. Taking into account real ratio of repeatitive and unique elements of human genome (95% to 5%) it is obvious that blood plasma DNA repertoire of this patient differs a lot from human genomic DNA repertoire. In this sample an abrupt enrichment by unique DNA sequences is observed.

For 15 out of 55 unique DNA fragments that were identified during sequencing of 96 clones from the library of blood plasma DNA, functions or product of correspondent gene were identified. Tables 1-15 present list of these sequences and information about their participation in formation and maintenance of "malignant phenotype".

Table 1

Clone	Product	Participation in oncogenesis	Source
Clone 24	Member of G protein – coupled receptor family	Playing major role in cancer cell signalling. Linked with cell transformation, supression of apoptosis, hormone independence and metastasis	Steeg P.S. ,Nat Rew Cancer,2003,v.3,pp .55-63. Raj G.V. ,J Urology,2002, v.167, pp.1458-1463. Hoff A.O., Neoplasia,1999 v.1, pp.485-491.

Table 2

Clone	Product	Participation in oncogenesis	Source
Clone 43	Snf2-coupled CBP activator protein (SCRAP)	Transription activator. Family members linked with synovial sarcoma and leukaemia	Thaete C., Hum Mol Genet,1999,v.8,pp. 585-91. Monroy M,A.,J

		development	Biol Chem,.2001,v.276, pp.40721-40726 Lee D.W., Cancer Res., 2000, v.60,pp.3612-3622.

Table 3

Clone	Product	Participation in oncogenesis	Source
Clone 51	SRY-box containing gene	Transcription modulator . Expressed during embryogenesis. Family members linked with medulloblastomas, gonadal tumors, highly metastatic melanoma.	Graham J.D., J Mol endocrinol, 1999,v.22,pp.295-304. Lee C.J.,J Neurooncol, 2002,v.57,pp.201-214. Uehara S., Cancer Genet Cytogenet,1999,v. 113.,pp.78-84. Tani M.,Genomics, 1997,v.39,pp.30-37

Table 4

Clone	Product	Participation in oncogenesis	Source
Clone 72	Protein- tyrosine kinase	Family members playing major role in cancer. Some PTK are cellular specific oncogenes products.	Hunter T., Philos Trans R Soc Lond B Biol Sci, 1998,v.353.,pp.583 -605. Scheijen B.,Oncogene, 2002,v.21.,pp.3314 -3333.

Table 5

Clone	Product	Participation in oncogenesis	Source
Clone 83	Fibroblast activation protein alpha; cell surface serine protease	Family members playing role in cancer invasion and metastasis. The product is active in cancer stroma and different carcinomas.	Chen W.T, Enzyme Protein,1996,v.49., pp.59-71. Scanlan M.J.,Proc Nat Acad Sci USA, 1994, v.91, pp.5657-5661. Mathew S., Genomics, 1995,v.25,pp.335-337.

Table 6

Clone	Product	Participation in oncogenesis	Source
Clone 86	Brain testican	Proteoglycan with unknown function. Linked with neoplastic phenotype of embryonal rhabdomyosarcoma cells.	Genini M., Int J Cancer, 1996,v.66,pp.571-577.

Table 7

Clone	Product	Participation in oncogenesis	Source
Clone 152	KRAB domain, Zn-finger proteins	Family members are known as transcription repressors. Linked with early embryogenesis, neuroblastoma, Ewing sarcoma, Tcell lymphoma, in progression and chemoresistance in lung cancer.	Oguri T.,Gene,1998,v.22 2,pp.61-67 Gou D.M.,Biochim Biophys Acta, 2001,v.1518,pp.30 6-310 Margolin J.F.,Proc Nat Acad Sci USA, 1994,v/91,pp.4509 -4513. Bellefroid E.J.,EMBO J, 1993,v.12,pp.1363

			-1374. Gonzales-Lamuno D.,Pediatr Pathol Mol Med, 2002,v.21,pp.531-540. Marilee J.W.,Gene, 1994,v.152,pp.227-232.

Table 8

Clone	Product	Participation in oncogenesis	Source
Clone 190	Antigen linked with melanoma	Antigen recognized by autologous tumor infiltrating lymphocytes.	J.Immunol.166(4), 2871-2877,2001

Table 9

Clone	Product	Participation in oncogenesis	Source
Clone 167	N-cadherin	Cell adhesion molecule with major role in cancer growth, invasion and metastasis.	Hazan R.B.,J Cell Biol, 2000,v.148,pp.779-790. Li G.,Cancer Res, 2001,v.61,pp.3819-3825. Tran N.L.,J Biol

			Chem, 2002,v.277,pp.329 05-32914.

Table 10

Clone	Product	Participation in oncogenesis	Source
Clone 197	FAF1: Fas associated factor 1	Phosphoprotein known to be the proapoptosis factor.	Jensen H.H.,Int J Biochem Cell Biol, 2001,v.33,pp.577-589. Ryu S.W.,Biochem Biophys Res Commun,

Table 11

Clone	Product	Participation in oncogenesis	Source
Clone 114	Interleukin 7	Proposed as essential paracrine \ autocrine growth factor for variety of cancers.	Trinder P., Int J Oncol, 1999,v.14,pp.23-31. Cosenza L.,Cell Signalling, 2002,v.14,pp.317-325.

Table 12

Clone	Product	Participation in oncogenesis	Source

Clone	DEAD Box RNA helicase – like protein	Family members involved to RNA methabolism. Linked to exponential cell growth in cancer.	Iggo R.D.,Mol Cell Biol, 1991,v.11,pp.1326 -1333. Causevic M.,Oncogene, 2001,v.20,pp.7734 -7743.
Clone 208			

Table 13

Clone	Product	Participation in oncogenesis	Source
Clone 97	Lipin 1	One of tumor cells' response regulators on cytotoxic compounds.	Brachat A. et.al., Oncogene, 2002,v.21,pp.8361 -8371

Table 14

Clone	Product	Participation in oncogenesis	Source
Clone 121	Dynein	Takes part in transport of p53 protein, is hypersecreted at cancer of prostate and hepatocellular cancer.	Bull J.H.,et.al.,Br J Cancer,2001,v.84,p p.1512-1519. Giannakakou P., et.al., Nat Cell Biol, 2000,v.2,pp.709-717 Jiang J.,et.al.,Gene,

			2001,v.281,pp.103 -113.

Table 15

Clone	Product	Participation in oncogenesis	Source
Clone 178	Ramp protein	Linked with human embrional carcinoma cells' development.	Cheung W.M., et.al., J Biol Chem,2001, v.276,pp.17083-17091

In this way 14 out of 15 sequences with identified function or protein that encodes different products (protein kinases, growth factors, proteinases, adhesive molecules and regulatory nuclear proteins) are described in literature as related to "malignant phenotype" formation and
5   maintenance. Only product of 197th clone identified as pro-apoptotic factor is not clearly linked with malignant progression. Though there is data concerning relationship between high apoptotic activity of tumor with its progression (Nishimura R., et al., J Surg Oncol,1999,v.71,pp.226-234) and possible role of apoptotic inductors in
10   formation and maintenance of immunosupression in malignant growth (O'Connel J., et al., Dis Esophagus,1999,v.12,pp.83-89).

The most significant presence from repeatitive elements in this material was alpha-satellite DNA (30 clones). It is possible to say that that alpha-satellite DNA was the only highly repeated element from
15   human genome, which behaves exactly as repeat in this material. The rest of highly repeatitive elements were presented in material as one or several clones (L1 variant and MLT26), or were not found among

patterns (MER, Alu, THE, MIR, ☐satellite). Based on today's knowledge one can assume that plasma blood composition for the most part should repeat composition of genome DNA, so listed repeats should be represented in the major part of clones while unique and moderately

5　repeated consequences in analysis of such a small number of clones should not be recognized at all. Received result clearly indicates on special way of plasma DNA formation in oncological patients. Another unexpected result is that of finding in the material of two new moderately repeated sequences – duplicones, that were recently

10　unknown, also support the evidence of special way of plasma DNA formation in patients with malignant tumor. For the first time duplicones were found in human genome less then two years ago. Known duplicones (Eichler E.E.,et al.,Genome Res,1998,v8,pp.791-808; Ji Y., et al.,Genome Res,2000,v.10,pp.597-610; Pujana M.A.,et al.,Genome

15　Res,2001,v.11,pp.98-111) are extensive regions of DNA that were multiplied for several times in the frame of one chromosome (unlike other repeats that are randomly allocated in genome).Duplicones' formation and expansion is connected with different genetic syndromes (for example Prader-Willi/Angelmane syndrome), with multigenic

20　families' evolution such as MHS (Shiina T., et al.,Proc Nat Acad Sci USA,1999,v.96,pp.13282-13287) and with chromosome instability in tumors.

It is necessary to mention that analysis of clones received from blood plasma DNA of patient has given us unexpected results.

25　Blood plasma DNA of oncological patient is highly enriched with unique genes. 55 out of 96 analyzed clones contain fragments of genome's unique sequences. 14 out of 15 sequences with identified in

functions refer to process of tumor progression and maintenance of "malignant phenotype".

Strong impoverishment of the most wide spread human repeat such as MER, Alu, THE, MIR, ⊓satellites is found in plasma DNA material.

Finding of two consequences with previously unknown duplicones' characteristics indicates on duplicones' representativeness in such DNA samples.

DNA library of healthy donor's blood plasma.

For method's value proof of blood plasma DNA cloning and sequencing for identification of genome's unique genetic consequences we have constructed DNA library of healthy donor's blood plasma. It is known that plasma of clinically healthy people also contains DNA but in significantly less amount than plasma of oncological patients (Shapiro B., et al., Cancer,1983,v.51,pp.2116-2120).

Representativeness of library was near 8×10^5 clones. We have got interesting result after analysis of 70 clones with length from 300 up to 1000 base pairs. We found out that 58 out of 70 analyzed clones are unique DNA sequences of human genome. After searching the HumanGenBank, we identified the function or product of correspondent gene for 14 out of 58 unique DNA fragments.

Only 12 clones contained fragments of repetitive sequences, herewith without of alpha-satellite DNA dominance.

So, it was unexpectedly found out that blood plasma DNA of healthy people and oncological patients for the most part contain unique fragments of human genome. In the case of oncological pathology unique sequences of blood plasma DNA correspond to genes which

products take part in the formation and maintenance of tumor cell's "malignant phenotype".

Basing on this unexpected discovery we have suggested that DNA circulating in patient's blood can be messenger of horizontal genetic information transfer during the course of oncological diseases, assisting to accumulation and spreading of genes that are necessary for "malignant phenotype" formation and maintenance whithin population of tumor cells.

Somatic mosaicism is a condition that is a result of genetically non-identical somatic cells' presence in organism. Modern vision presents that many non tumor and noninfectious (so called somatic) human diseases (for example atherosclerosis, diabetes, nonspecific chronic lung diseases and so on), including aging process, are connected with appearance and spreading (expansion) in the process of individual development of somatic cells' clones that have mutant genes. (Youssoufian H., et al., Nature Rew. Genet., 2002,v.3,pp.748-758; J.Vijg, Mutation Res.,2000,v.447,pp.117-135; R.Erickson, Mutation Res.,2003,v.543,pp.125-136;   Andreassi   M.,Mutation Res.,2003,v.543,pp.67-87;Anderson   G.,   et   al.,   Trends   in Pharmacological Sci.,2003.,v.24,pp.71-76).

Bright example of such process is progression of mitochondrial heteroplasmia (expansion of mutant mitochondrial DNA) at different diseases and in the aging process (E.Jazin et al., Proc Nat Acad Sci USA, 1996, v.93,pp.12382-12387; Michikawa Y. et al., Science, 1999, v.286, pp.774-779;Calloway C. et al., Am J Hum Gen,2000,v.66,pp.1384-1397).

There are two alternative models of somatic mosaicism's appearance. The first is appearance of somatic mosaicism as a result of numerous "de novo" mutations in polyclonal cellular pool. The second model is clonal expansion of mutant cells' clone (Khrapko K., et al.,

5   Muation Res.,2003,v.522,pp.13-19).

In the process of work above the invention we have found that DNA circulating in the blood of healthy people play significant role in somatic mosaicism's development and its binding, destruction or inactivation inhibits development of somatic mosaicism. Binding,

10   destruction or inactivation of circulating in blood plasma DNA provides treatment effect at diseases which appearance is connected with somatic mosaicism's development.

Examples that are mentioned later indicates role of circulating in blood of oncological patients DNA in the development of tumor's

15   resistance to chemotherapy, development of metastasing process, in sepsis development and in some other pathological conditions. High therapeutic effect of blood plasma DNA's binding, destruction or inactivation is found.

Role of free circulating DNA in tumor progression

20   Materials and methods.

Bovine pancreatic DNase I (Fermentas) and human recombinant DNase I (Dornase; Gentech) were used. DNase I solution for injections were prepared by dissolving of DNase matrix solution in sterile phosphate buffer directly before administration. Cyclophosphamide and

25   Doxorubicin were dissolved according to instruction.

In series of in vitro experiments we have not seen any supressive effect of DNase I on tumor cells' growth (DNase I concentration was up to 100 mkg/ml).

Blood plasma DNA of tumor-bearing mice was received according to technique described above. Highly metastatic and low metastatic stains of mice' Luice lung carcinoma and Erlich carcinoma were used. Cells were cultivated in RPMI-1640 media with adding of 10% embryonic calf serum, 1% penicillin – streptomycin in 5% carbon dioxide atmosphere. For tumor induction in mice, cells were cultivated until the monolayer , than were detached with trypsin-EDTA solution . Cells were washed trice by centrifuging in phosphate buffer and were resuspensed until their concentration have become $0{,}5 \times 10^7$/ml. Viability was defined by inclusion of methylene blue in hemocytometer. Suspensions that contained 95% of viable cells were used for administration to animals.

Mice of C57B1 line and white not thoroughbred mice from "Rappolovo" animal house were used. Weight of animals was 24-26 grams. Animals were contained in cages, for 6-7 mice per one cage on a standard diet without water restriction. LLC cells in dose of 5×10^5 in 100 mkl of phosphate buffer were injected in soft tissues of thigh. Erlich tumor was subcutaneously replanted on the right side by injection of 0,2 ml 10% cells' suspension in isotonic sodium chloride solution.

In some experiments DNA presence in blood plasma of mice was studied.

DNA was isolated by method that is mentioned above. DNA amount was spectrophotometrically measured.

The invention is illustrated by following examples.

Example 1.

Influence of DNase I administrated twice a day on Erlich tumor growth at mice.

Group 1 – 10 mice transplanted with Erlich carcinoma (control).

Group 2 – 10 mice transplanted with Erlich carcinoma. Mice were intraperitoneally injected with DNase at 1 mg/kg dose in 200 ml of phosphate buffer twice a day starting from 3 up to 7 day after tumor transplantation.

Group 3 – 10 mice transplanted with Erlich carcinoma. Mice were intraperitoneally injected with DNase at 2 mg/kg dose in 200 ml of phosphate buffer twice a day starting from 3 up to 7 day after tumor transplantation.

.Results of experiments were estimated according to tumor's growth inhibition (TGI) that were expressed in per cents to control data received at last day of DNase injection with standard formula using.

Tumor's size on the 7 day after transplantation.

Group	Tumor's volume	TGI%	P
1	86+/-12	-	-
2	33+/-6	61%	p<0,001
3	34+/-7	60%	p<0,001

The data obtained indicates that tumor's growth is significantly inhibited by DNase injection.

Example2

Inhibition of Erlich tumor growth by use of different schemes of DNase administration.

In our experiments DNA circulating in blood plasma of patients was the therapeutic target of DNase. Prolonged presence of DNase in blood plasma at catalytically effective concentrations is essential for provision of maximal therapeutic effect. According to that DNase myltiply administration should provide better therapeutic effect than the same total daily dose, administrated just as only two daily injections.

Group1 – 10 mice transplanted with Erlich carcinoma (control).

Group 2 – 10 mice transplanted with Erlich carcinoma. Mice were intraperitoneally injected with DNase at 1 mg/kg dose in 200 ml of phosphate buffer twice a day starting from 3 up to 7 day after tumor transplantation.

Group 3 – 10 mice transplanted with Erlich carcinoma. Mice were intraperitoneally injected with DNase at 0,5 mg/kg dose in 200 ml of phosphate buffer four times a day starting from 3 up to 7 day after tumor transplantation.

.Results of experiments were estimated according to tumor's growth inhibition (TGI) that were expressed in per cents to control data received at last day of DNase injection with standard formula using

Tumor's size on the 7 day after transplantation.

Group	Tumor's volume	T%	P
1	98+/-14	-	-
3	23+/-6	76%	P<0,001
2	32+/-6	67%	P<0,001

Received data indicates that tumor's growth is more inhibited by fractional (four times a day) administration of DNase at the same total daily dose than twice a day administration. Blood plasma DNA concentration of mice from 3 group after end of treatment course was

38,5 ng/ml, mice from control group had 104,8 ng/ml and mice from the 2 group – 55,1 ng/ml. Blood plasma DNA concentration of healthy mice was 12,0 ng/ml ($p<0,01$). So, multiply daily administrations of DNase lead to more significant decrease of blood plasma DNA level and greater tumor's growth inhibition in comparison with twice a day administration of the same total dose.

Example 3

Combined use of DNase and antitumor Doxorubicin compound.

Group 1 – 10 mice transplanted with Erlich carcinoma (control).

Group 2 – 10 mice replanted with Erlich carcinoma. Mice were intravenously injected with Doxorubicin at 2 mg/kg dose once a day starting from 3 up to 7 day after tumor transplantation.

Group 3 – 10 mice replanted with Erlich carcinoma. Mice were intravenously injected with Doxorubicin at 2 mg/kg dose once a day starting from 3 up to 7 day after tumor transplantation. As well mice were intraperitoneally injected with DNase at 0,5 mg/kg dose in 200 ml of phosphate buffer four times a day starting from 3 up to 7 day after tumor transplantation.

Group 4 – 10 mice replanted with Erlich carcinoma. Mice were intraperitoneally injected with DNase at 0,5 mg/kg dose in 200 ml of phosphate buffer four times a day starting from 3 up to 7 day after tumor transplantation.

.Results of experiments were estimated according to tumor's growth inhibition (TGI) that were expressed in per cents to control data received at last day of DNase injection with standard formula using.

Tumor's size on the 7th day after transplantation.

Group	Tumor's volume	T%	P
1	98+/-14	-	-
2	57+/-10	42	P<0,05
4	20+/-6	78	P<0,01
3	0	100%	

Received data indicates that tumor's growth is less inhibited by Doxorubicin than by DNase. Combined use of Doxorubicin and DNase present synergism in their action leading to complete inhibition of

5   tumor's growth (tumors were not found in any experimental animals).

Example 4

Influence of DNase on bacterial biofilm formation.

For estimation of possible mechanisms of DNase action on bacteria' interactions with host's organism we have estimated its

10   influence on biofilm formation.

Experiments were performed on biofilm models, received at laboratory cultivation on the surface of glass. Non-relative Gram-positive (Staphylococcus aureus VT-209) and Gram-negative (Escherichia coli ATCC25922) bacteria were used.

15   Bacteria were sowed at 10^9 bacteria/ml concentrations and were incubated at 37^0C during 72 hours on synthetic M9 media that contains additives necessary for used bacteria strains. DNase was added in amount of 100 mkg/ml right after bacteria' inoculation. Identical volume of phosphate buffer was added to control. Selective vials were taken for

20   analysis every 24 hours.

Glasses were washed by PBS buffer, fixed, stained with methylene blue and studied with the use of light microscopy.

As a result it was found out that in DNase presence normal biofilm was not formed. Only adhesion of single microorganisms to the glass that have not lead to the formation of microcolonies and biofilms was observed.

5   Control sowings from vials for definition of colony-forming units(CFU) have shown that at DNase presence there is no death of cells leading to decrease of **CFU**'s numbers in comparison with controls. Received data indicates that DNase do not cause death of Staphylococci and Escherichia, but prevent their cooperative behavior that leads to

10   biofilm formation.

Example 5

DNase' use for treatment of the experimental sepsis.

White not thoroughbred mice were used for experiment. Animals' weight was 24-26 grams. Pathogenic strain of Staphylococcus aureus

15   VT-2003R at 1×10^{10} bacteria/animal dose was inoculated to retroorbital sinus of animals. DNase was intraperitoneally administred. Isotonic solution of sodium chloride or Penicillin was administrated to control groups. Each experimental group contained 10 mice. Intraperitoneal administration of compound (DNAase) four times a day at 500 mkg/kg

20   dose continued for 1-3 days after infection. Penicillin was intramusculary administrated. Efficiency of action was estimated by number of animals that stayed alive after the death of the last one in control group. All animals have died to the third day of the experiment in control group. Six animals stayed alive to the third day of the

25   experiment in the group that administrated DNase. Protection was 67%. Received data specify an opportunity and efficiency of DNase use for infections' treatment.

Example 6

Level of free circulating plasma DNA at oncological patients and healthy adults.

Blood plasma DNA of oncological patients and blood plasma DNA of healthy donors differs not only by its genetic repertoire but also by its amount circulating in blood plasma. Table below presents data of blood plasma DNA contents at 10 oncological patients and at 10 healthy volunteers. DNA isolation from plasma and determination of its amount was done according to protocol mentioned above.

Patient	Sex, age	Tumor, stage	DNA contents; ng\ml
1	M, 67	Lung carcinoma T2N2M0	123
2	F,37	Breast cancer T2N0M0	78
3	F,53	Gaster carcinoma T3N2M1	90
4	M,54	Cancer of large intestine T2N2M2	340
5	M,64	Cancer of large intestine T2N1M0	182
6	M,56	Lung carcinoma T3N2M1	99
7	F,49	Cancer of large intestine T2N1M0	75
8	M,65	Gaster carcinoma T3N1M0	120

9	M,36	Osteogenic sarcoma T3N1M2	243
10	F,50	Breast cancer T3N1M0	165
11	M,24	Volunteer	10
12	M,32	Volunteer	27
13	F,21	Volunteer	45
14	F,19	Volunteer	7
15	F,21	Volunteer	13
16	F,23	Volunteer	89
17	M,28	Volunteer	11
18	F,32	Volunteer	15
19	F,25	Volunteer	17
20	M,38	Volunteer	5

As it is clear from the table the level of volunteers' blood plasma DNA is considerably lower than in blood of patients with different malignant neoplasms.

Example 7

DNA clones' sequences, received from free circulating blood plasma DNA of patient with malignant mesothelioma.

Clone   1

Duplicon, chromosome 15 and Y

Sequence №1.

Clone 3

Unique,chromosome 2.

Sequence №2

Clone 8

MLT2B repeat

Sequence №3

Clone 9

Centromeric satellite DNA

5   Sequence №4

Clone 10

MLT2B repeat

Sequence №5

Clone 20

10   L1MC4-like (LINE-element)

Sequence №6

Clone 15

Alpha-satellite DNA

Sequence №7

15   Clones 18, 21

Alpha-satellite DNA

Sequence №8

Clone 24

Unique, family of G protein-bound proteins, chromosome 6.

20   Sequence №9

Clone 25

Unique, chromosome 3.

Sequence №10

Clone 26

25   SatB1/Vimentin/nuclear matrix binding DNA

Sequence №11

Sequence 33

Duplicon specific to the chromosome 10

Sequence №12

Clone 32

alpha-satellite DNA

5   Clone 35

LTR repeat

Sequence №13

Clone 36

Unique, chromosome 18

10   Sequence №14

Clone 37

Unique, chromosome 4

Sequence №15

Clone 41

15   Sequence №16

Clone 43

Snf2-related CBP activator protein (SCRAP)

Unique, chromosome 16

Sequence №17

20   Clone 45

Unique, chromosome 3

Sequence №18

Clone 47

Alpha-satellite DNA

25   Clone 51

SRY-box containing gene.

Sequence №19

Clone   52

Repeat

Sequence №20

Clone 53, 55

5   Alpha-satellite DNA

Sequence №21

Clone 56

Centromeric repeat

Sequence №22

10   Clone 60

Gene repeated on several chromosomes, contains MER5A repeat.

Sequence №23

Clone 62

Repeat

15   Sequence №24

Clone 65

Unique, chromosome 2

Sequence №25

Clone 71

20   Unique, chromosome 2

Sequence №26

Clone 72

Unique, chromosome 8

Sequence №27

25   Clone 73

Unique

Sequence №28

Clone 78

Transposon Tigger fragment

Sequence №29

Clone 81

5   Sequence №30

Repeat (LINE)

Clone 82

Unique, chromosome 1

Sequence №31

10   Clone 83

Unique, Fibroblast activation protein alpha; cell surface serine

protease

Chromosome 2

Sequence №32

15   Clone 79

Alpha-satellite DNA

Clone   86

Unique, gene highly similar to brain testican , chromosome 4.

Sequence №33

20   Clone 90

Unique, chromosome X

Sequence №34

Clone 93

Unique, chromosome 9

25   Sequence №35

Clones 89 и 92

Alpha-satellite DNA

Clone 96

Fragment LINE.

Sequence №36

Clone 97

5   Chromosome 2 unique, Lipin

Clone 98

Unique, chromosome X

Sequence №38

Clone 102

10   Chromosome 17 unique

Sequence №39

Clone 99

Alpha-sattelite DNA

Clone105

15   Unique, chromosome 13

Sequence №40

Clone N106

Chromosome 9 unique

Sequence №41

20   Clone 107

Unique, chromosome 8

Sequence №42

Clone N 111

Unique, chromosome 12

25   Sequence №43

Clone N 112

Chromosome 5 unique

Sequence №44

Clone 114

Chromosome 8 unique; Interleukin 7

Sequence №45

5   Clone 116

Chromosome 1 unique

Sequence №46

Clone 121

Chromosome 5 unique; Dynein

10   Sequence №47

Clone 115; 119;120

Alpha-sattelite DNA

Clone 125

Chromosome 9 unique

15   Sequence №48

Clone127

Unique chromosome 20

Sequence №49

Clone 130

20   Unique, chromosome is not determined.

Sequence №50

Clone124

SatB1/Vimentin/nuclear matrix binding DNA

Clone 133

25   Alpha-satellite DNA

Clone 137

MLT1A2 repeat

Sequence №51

Clone 140

Unique, chromosome 2 ;zinc finger protein, subfamily 1A

Sequence №52

5   Clone 141

Chromosome 2 unique

Sequence №53

Clone143

Fragment of Alu-repeat

10   Sequence №54

Clone 144

Chromosome 2 unique

Sequence №55

Clone 146

15   Chromosome 4 unique

Sequence №56

Clone 139 and 142

Alpha-sattelite DNA

Clone 148

20   Repeat (chromosomes 1,2 and 4)

Sequence №57

Clone152

Unique, chromosome 16;KRAB-Domain, zinc finger protein

Sequence №58

25   Clone 154

Chromosome 9 unique

Sequence №59

Clone 161

Fragment LINE

Sequence №60

Clone 151

5   Chromosome 5 unique

Sequence №61

Clone 150

Chromosome 1 unique

Sequence №62

10   Clone 153

Chromosome 11 unique

Sequence №63

Clone159

Chromosome 6 unique

15   Sequence №64

Clone 163

Alpha satellite DNA

Sequence №65

Clone 166

20   Chromosome 12 unique

Sequence №66

Clone 167

Unique , chromosome 18, CDH2; cadherin 2, type 1, N-cadherin

Sequence №67

25   Clones 169, 170

Chromosome 18 unique

Sequence №68

Clone 178

Unique chromosome 1; RAMP: RA-regulated nuclear matrix-associated protein

Sequence №69

5   Clone 180

Unique, chromosome 20

Sequence №70

Clone 181

Unique chromosome 18

10   Sequence №71

Clone 185

Alpha-satellite DNA

Sequence №72

Clone 187

15   Mer repeat

Sequence №73

Clone 188

HSATII repeat

Sequence №74

20   Clone 189

Chromosome 9 unique

Sequence №75

Clone 190

Chromosome 1 unique; melanoma antigen recognized by T cells 2

25   Sequence №76

Clone 195

Chromosome 10 unique

Sequence №77

Clone 196

Chromosome X unique

Sequence №78

5   Clone 197

Chromosome 1 unique, FAF1: Fas (TNFRSF6) associated factor 1

Sequence №79

Clone 200

Chromosome 8 unique

10   Sequence №80

Clone 202

Unique chromosome 13

Sequence №81

Clone 205

15   Alpha satellite DNA

Sequence №82

Clone 206

Repeat

Sequence №83

20   Clone   208

Unique chromosome 8;Human DEAD box RNA helicase-like

protein

Sequence №84

Example 8

25   DNA clones sequences received from free circulating blood

plasma DNA of healthy donor.

Clone 1

Chromosome 5 unique

Sequence №85

Clone 9

5   Unique chromosome 21

Sequence №86

Clone 7

Unique chromosome 3

Sequence №87

10   Clone 8

Chromosome 4 unique

Sequence №88

Clone10

18S RNA gene

15   Sequence №89

Clone11

Alu repeat

Sequence №90

Clone13

20   Unique chromosome 3

Sequence №91

Clone15

Unique chromosome 1

Sequence №92

25   Clone 16

Unique chromosome 3, neutral endopeptidase

Sequence №93

Clone 17

Chromosome 8 unique

Sequence №94

Clone 18

5   Chromosome 1 unique

Sequence №95

Clone 21

Unique chromosome 19;Zinc Finger protein

Sequence №96

10   Clone 22

Unique chromosome 18

Sequence №97

Clone 23

Unique chromosome 7, muskelin 1

15   Sequence №98

Clone 25

Unique chromosome 11

Sequence №99

Clone 27

20   Repeat

Sequence №100

Clone 29

Unique chromosome 6

Sequence №101

25   Clone 30

Unique chromosome 14

Sequence №102

Clone 31

Unique chromosome 17

Sequence №103

Clone 32

5   MER4B repeat

Sequence №104

Clone33

Chromosome 1 unique

Sequence №105

10   Clone 34

Unique chromosome 2

Sequence №106

Clone 35

Repeat

15   Sequence №107

Clone 36

Chromosome 1 unique

Sequence №108

Clone 37

20   HERVH repeat

Sequence №109

Clone 41

Chromosome X unique

Sequence №110

25   Clone 42

Chromosome 6 unique

Sequence №111

Clone 43

Unique chromosome 22; KREMEN1

Sequence №112

Clone 44

5   Unique chromosome 14

Sequence №113

Clone 45

Unique

Sequence №114

10   Clone 46

Chromosome 20 unique

Sequence №115

Clone 47

Nf-kappaB

15   Sequence №116

Clone 38

Unique chromosome 16

Sequence №117

Clone 48

20   Chromosome 6 unique

Sequence №118

Clone 53

Unique

Sequence №119

25   Clone 51

Chromosome 5 unique

Sequence №120

Clone 59

Unique chromosome 4, NFKB1: nuclear factor of kappa light
polypeptide gene enhancer

Sequence №121

5   Clone 61

Repeat

Sequence №122

Clone 62

L1 repeat

10   Sequence №123

Clone 64

Duplicon chromosome 7

Sequence №124

Clone 65

15   Ribosomal DNA

Sequence №125

Clone 66

Rbosomal DNA

Sequence №126

20   Clone 75

Repeat

Sequence №127

Clone 76

Chromosome 4 unique

25   Sequence №128

Clone 83

Chromosome 4 unique

Sequence №129

Clone 85

Unique chromosome 2; phospholipase C, epsilon

Sequence №130

5   Clone 87

L1PA3 repeat

Sequence №131

Clone 86

Unique chromosome 5; CRTL1: cartilage linking protein 1

10   Sequence №132

Clone 89

Alu repeat

Sequence №133

Клон 92

15   Unique chromosome 6

Sequence №134

Clone100

Unique, chromosome 6

Sequence №135

20   Clone 105

AluSx repeat

Sequence №136

Clone 111

Alphoid repetitive DNA

25   Sequence №137

Clone 112

Chromosome 9 unique

Sequence №138

Clone 113

Chromosome 22 unique

Sequence №139

5   Clone 114

AluSx repeat

Sequence №140

Clone 116

Unique chromosome 9; 17kD fetal brain protein

10   Sequence №141

Clone 123

Unique chromosome 5

Sequence №142

Clone 124

15   Unique chromosome 13

Sequence №143

Clone 126

Unique chromosome 8

Sequence №144

20   Clone 130

Unique chromosome 1

Sequence №145

Clone 131

Unique chromosome 4

25   Sequence №146

Clone136

Unique chromosome 8

Sequence №147

Clone 141

Unique chromosome 2

Sequence №148

5   Clone 146

Unique chromosome 16

Sequence №149

Clone 147

Unique chromosome 5; nicotinamide nucleotide transhydrogenase

10   Sequence №150

Clone149

Unique chromosome 9

Sequence №151

Clone 151

15   Unique chromosome 16

Sequence №152

Clone 152

Unique chromosome 6, BAI3: brain-specific angiogenesis

inhibitor 3

20   Sequence №153

Clone 153

Unique chromosome 9, GAD2: glutamate decarboxylase 2

Sequence №154

Clone 155

25   Unique chromosome 9

Sequence №155

Example 9

Sensitivity of circulating blood DNA to DNase action.

DNA from fresh blood serum (mixture of blood received from 5 volunteers) was isolated by standard phenol-chloroform method.

5   Sediment of nucleic acids was washed by 70% ethanol and dissolved in tris-EDTA buffer. Spectrophotometry with wave's length 260 nm (SF-46) was used for quantification of DNA. DNA was researched by electrophoresis in 0,8% polyacrylamide gel with ethidium bromide staining. One part of received DNA was processed by DNase I 10   for 3 hours at 37^0C. After electrophoretic separation of processed and not processed DNA it was found out that:

After electrophoresis non-processed DNA constitute one compact band. This factor indicates on relatively identical size of DNA circulating in blood. Said band disappeared completely after DNAse 15   processing. Received data indicates that DNA circulating in serum is sensitive to DNases use and can be destroyed by their action.

Example 10

Results of the experiment of influence of polyclonal serum containing anti-DNA antibodies' on life span of Erlich carcinoma 20   bearing mice.

DNA circulating in blood plasma of oncological patients can be destroyed or inactivated not only by DNA – digesting enzymes (for example by DNase), but also by other methods.

Anti-DNA antibodies were isolated from blood of patients with 25   lupus erythematosus by Shuster A.M. method (Shuster A.M. et.al.,Science,v.256,1992,pp.665-667). Such anti-DNA antibodies were capable not only to bind DNA but also to hydrolyze it.

Group 1 – 7 mice transplanted with Erlich carcinoma (control).

Group 2 – 6 mice transplanted withy Erlich carcinoma. Mice were injected intravenously with fraction of human anti-DNA antibodies (Ig G) at 200 mkg dose on the third day after tumor transplantation.

5 Group 3 – 6 mice transplanted withy Erlich carcinoma. Mice were injected intravenously with fraction of human non specific immunoglobulin (Ig G) at 200 mkg dose on the third day after tumor transplantation.

Effect was estimated according to tumor's growth inhibition on 10 the 7th day after transplantation (TGO expressed in per cents).

Tumor's on the 7th day after transplantation.

Group	Tumor's volume	T%	P
1	85+/-12	-	-
2	45+/-6	52%	p<0,001
3	79+/-7	7%	p<0,001

Results of experiments indicates that single anti-DNA antibodies' administration possess strong antitumor effect. Administration of antibodies fraction that does not contain ant-DNA antibodies do not 15 possess antitumor activity.

Example 11

Effect of mice' vaccination by blood plasma DNA fraction received from animals with Erlich carcinoma on transplantation and growth of Erlich carcinoma at immunized animals.

20 DNA from plasma of mice with Erlich carcinoma was isolated on the 5th day after transplantation according to method mentioned above. Positively charged multilayer liposomes were used as adjuvants for

immunization. DNA sample was mixed with liposomes (20 mkg of DNA in 1 mg of lipids).

Group 1 – 6 non immunized mice.

Group 2 – 6 mice immunized for three times with one week interval by intradermal administration of 1 mkg DNA from blood plasma in 50 mkl of liposomal suspension containing 50 mkg of liposomes.

Group 3 – 6 mice immunized for three times with one week interval by intradermal administration of 50 mkl of liposomal suspension containing 50 mkg of liposomes without DNA.

Group 4 – 6 mice immunized for three times with one week interval by intradermal administration of 1 mkg DNA from calf thymus (Sigma) in 50 mkl of liposomal suspension containing 50 mkg of liposomes.

One week after the last immunization Erlih carcinoma was transplanted to all mice including non immunized control mice.

Results of the experiment were estimated according to animal survival on the 30th and 50th day after tumor's transplantation.

Group	30 day (number of alive\ number of dead in group)	50 day (number of alive\ number of dead in group)
1	0-6	0-6
2	5-6	3-6
3	0-6	0-6
4	2-6	0-6

So immunization of mice with replanted Erlich carcinoma by blood plasma DNA lead to significant increasing of animals' lifetime.

Example 12

DNA isolation from biofilm matrix formed by Gram-positive and Gram-negative bacteria.

Biofilms of Escherichia coli and Staphylococcus aureus were used in experiments. Biofilms were washed off by phosphate buffer from agar. Cells and matrix were separated by centrifuging. Standard phenol-chloroform method was used for DNA extraction from matrix. DNA amount was estimated by spectrophotometric analysis with wave's length 260 nm (SF-46). Received DNA was researched by electrophoresis in 0,8% polyacrylamide gel with ethidium bromide staining.

One part of received DNA was processed for 3 hours by DNAase I at 37^0C. After electrophoretic separation of processed and not processed DNA it was found out that:

After electrophoresis non-processed DNA form one compact line. This fact indicates relatively identical size of DNA in biofilm matrix. Said band disappeared completely after DNAse processing. So the biofilm matrix of gram positive and gram negative bacteria contains extracellular DNA, which could be destroyed with DNAse.

Example 13

Dynamics of P-glycoprotein expression in Erlich carcinoma at mice that receive Doxorubicin therapy and DNase I effect.

Treatment by Doxorubicin causes expression of P-glycoprotein in tumor tissue that is one of the main MDR (Multidrug Resistance) phenotype mediators. Immunohistochemical staining of mice's tumor histological cuts are listed below.

Mice were subjected to course of 5 day therapy with Doxorubicin (2 mg/kg intravenously daily) or Doxorubicin + DNase I (0,5 mg/kg four times a day during 5 days)

Treatment has begun on the 3rd day after tumor's transplantation.

5 Tissue preparations were executed on the 8th day of tumor's transplantation. Multifocal expression of P-glycoprotein was observed in tumor tissue after 5 days of therapy (Fig.1).

Total level of P-glycoprotein expression and amount of P-glycoprotein positive nodules in tumor tissue was much lower at the case

10 of combined treatment by Doxorubicin + DNase (Fig.1). So treatment by DNase delays development of multidrug resistant phenotype in tumor that is caused by antitumor antibiotic Doxorubicin use.

Example 14

Influence of plasma DNA of C57Bl mice with LLC tumor after

15 chemotherapeutical treatment by Doxorubicin, on LLC tumor growth and metastasizes development at C57Bl mice receiving Doxorubicin therapy and effect of DNase I .

LLC tumor was replanted to 30 C57Bl mice. Twenty mice were treated with Doxorubicin at 2 mg/kg dose daily for 5 days, starting day 3

20 after transplantation. Ten mice were treated with Cyclophosphamide at 15 mg/kg dose intraperitoneally for once on the 3rd day after replantation. Such treatment scheme does not lead to animal's recovery but leads to 50 % tumor inhibition at day 8 in doxorubicin treated animals and 30% tumor inhibition at day 8 in Cyclophosphamide treated

25 animals. On the next day after end of chemotherapy course animals were euthanized and and total blood plasma from both mice groups was taken.

After isolation total fraction of blood plasma DNA was stored at -20^0C in phosphate buffer.

Five groups of mice that were transplanted with LLC tumor participated in experiment.

Group 1 – 7 mice (control).

Group 2 – 6 mice intravenously treated with Doxorubicin chemotherapy from 3rd up to 8th day at 2 mg/kg dose daily.

Group 3 – 6 mice intravenously treated with Doxorubicin chemotherapy from 3rd up to 8th day at 2 mg/kg dose daily + intravenous administration of DNA fraction from mice previously subjected to Doxorubicin chemotherapy (0,05 mkg of DNA in 200 mkl of phosphate buffer at day 1 and day 3 after initiation of treatment)

Group 4 -6 mice intravenously treated with Doxorubicin chemotherapy from 3rd up to 8th day at 2 mg/kg dose daily + intravenous administration of DNA fraction from mice previously subjected to cyclophosphamide chemotherapy (0,05 mkg of DNA in 200 mkl of phosphate buffer at day 1 and day 3 after initiation of treatment)

Group 5 – 6 mice intravenously treated with Doxorubicin chemotherapy from 3rd up to 8th day at 2 mg/kg dose daily + intravenous administration of DNA fraction from mice previously subjected to Doxorubicin chemotherapy (0,05 mkg of DNA in 200 mkl of phosphate buffer at day 1 and day 3 after initiation of treatment) + intraperitoneal administration of DNase I at 0,5 mg/kg dose for 4 times a day at the first and second days of treatment.

Tumor's size on the 8th day after transplantation.

Group	Tumor's size
1	127+/-13
2	67+/-7
3	115+/-20
4	75+/-11
5	82+/-9

So administration of blood plasma DNA from mice subjected to chemotherapy lead to tumor's resistance to chemotherapeutic treatment. DNase's administration prevents appearance of this effect.

Example 15

Influence of blood plasma DNA from C57Bl mice with highly metastatic LLC strain on metastasizing of low metastatic LLC tumor strain in C57Bl mice and effect of DNase I.

LLC tumor was transplanted to 30 C57Bl mice. Twenty mice were transplanted with highly metastatic strain and 10 mice were transplanted with low metastatic strain. On the 9th day animals were euthanized and total blood plasma of both mice groups was collected. After isolation the total fraction of blood plasma DNA was stored at -20^0C in phosphate buffer.

Five groups of mice with transplanted LLC tumor participated in the experiment.

1 Group – 6 mice transplanted with low metastatic LLC strain.

2 Group – 6 mice transplanted with low metastatic LLC strain + intravenous administration of total DNA fraction from mice with transplanted highly metastatic strain (0,05 mkg of DNA in 200 mkl of phosphate buffer on the 7th and 8th day after transplantation) .

3 Group – 6 mice transplanted with low metastatic LLC strain + intravenous administration of total DNA fraction from mice with transplanted low metastatic strain (0,05 mkg of DNA in 200 mkl of phosphate buffer on the 7th and 8th day after transplantation)

4 Group – 6 mice transplanted with low metastatic LLC strain + intravenous administration of total DNA fraction from mice with transplanted higly metastatic strain (0,05 mkg of DNA in 200 mkl of phosphate buffer on the 7th and 8th day after transplantation) + intraperitoneal administration of DNase I at 1 mg/kg dose two times daily at 7th and 8th day after transplantation.

5 Group – 6 mice transplanted with highly metastatic LLC strain.

Number of metastatic foci in lungs was estimated bon the 15th day after transplantation (N).

Experiments' results are presented in the table.

Group	N
1	12,0
2	24,1
3	14,6
4	11,6
5	33,6

Received data indicates that blood plasma DNA from mice with highly metastatic LLC strain intensify metastasizing of low metastatic LLC strain.

DNase administration prevents appearance of this effect.

Example 16

DNase I influence on life span of C57Bl mice transplanted with LLC tumor (highly metastatic strain).

Five groups of LLC transplanted mice participated in the experiment.

Group 1 – 7 mice (control).

Group 2- 6 mice were treated with intraperitoneal administration

5 of DNase at 1 mg/kg dose two times a day starting from 3 up to 7 day after tumor transplantation.

Group 3 – 6 mice were treated with intraperitoneal administration of DNase at 1 mg/kg dose two times a day starting from 3 up to 10 day after tumor transplantation.

10 Group 4 – 6 mice were treated with intraperitoneal administration of DNase at 1 mg/kg dose two times a day starting from 3 up to 15 day after tumor transplantation.

Group 5 – 6 mice were treated with intraperitoneal administration of DNase at 1 mg/kg dose two times a day starting from 3 up to 18 day

15 after tumor transplantation.

Results of experiment were estimated according to animals' survival on the 30 and 50 day after tumor transplantation.

Group	30 day (number of alive\ number of dead in group)	50 day (number of alive\ number of dead in group)
1	0-7	0-7
2	0-6	0-6
3	3-6	0-6
4	5-1	3-3
5	6-0	6-0

The significant inhibition of tumor's growth was observed at the last day of DNase treatment in the 2nd and 3rd groups, but tumor's growth renewed after DNase withdrawal and to the 25th day size of tumor in this groups and in control has equalized.

The most longitudinal course o DNase treatment (from 3rd up to 18th day – group number 6) has lead to maximal survival. Inhibition of tumor growth was more than 95% at day 18.

In all experiments single and multiple injection of up to 2,5 mg/kg of human DNase I (maximal dose that was used in experiments) had no toxic effect on animals.

So, DNase I does not cause direct cytotoxic effect on tumor cells (in our in vitro experiments at concentration of 100 mkg/ml) and experimental data confirm that antitumor effect is connected with destruction of DNA in blood plasma and DNase's therapeutic effect increases with increasing of its treatment course duration.

Example 17

Influence of different methods of blood plasma DNA's destruction, inactivation, and binding on ability of blood plasma DNA from C57Bl mice with transplanted highly metastatic LLC strain to intensify metastasizing of low metastatic LLC tumor strain in C57Bl mice.

100 mice were transplanted with highly metastatic LLC strain. On the 9th day after transplantation, animals were euthanized and total blood plasma was taken. After isolation total fraction of blood plasma DNA was stored at -20^{0}C in phosphate buffer.

Six groups of mice with transplanted low metastatic LLC strain participated in the experiment .

Group 1 – 6 mice transplanted with low metastatic LLC strain.

Group 2 – 6 mice transplanted with low metastatic LLC strain +
two intravenous injections of total DNA fraction from mice transplanted
with highly metastatic strain on the 7th and 8th day after

5   transplantation(0,05 mkg of DNA was dissolved in 500 mkl of fresh
heparinized blood before injection).

Group 3 - 6 mice transplanted with low metastatic LLC strain +
two intravenous injections of total DNA fraction from mice transplanted
with highly metastatic strain on the 7th and 8th day after

10   transplantation(0,05 mkg of DNA was dissolved in 500 mkl of fresh
blood plasma before injection). Before administration the sample was
photochemically disinfected (1 mkM of methylene blue was added with
following irradiation by red light during 10 minutes (60 000 Lux).

Group 4 – 6 mice transplanted with low metastatic LLC strain +

15   two intravenous injections of total DNA fraction from mice transplanted
with   highly   metastatic   strain   on   the   7th and   8th day   after
transplantation(0,05 mkg of DNA was dissolved in 500 mkl of fresh
blood plasma before injection). The sample was passed through the
column containing DEAE-cellulose for two times before administration.

20   Group 5 - 6 mice transplanted with low metastatic LLC strain +
two intravenous injections of total DNA fraction from mice transplanted
with   highly   metastatic   strain   on   the   7th and   8th day   after
transplantation(0,05 mkg of DNA was dissolved in 500 mkl of fresh
heparinized blood before injection . 1 mkg of fragment A of Ricin toxin

25   was added to the sample before administration and sample was incubated
at 370C for 1 hour. Ricin toxin is representative of RIP toxins family
(proteins that inactivate ribosomes) which are used for immunotoxin's

creation. Besides their ability to inactivate ribosomes these proteins can **deadenylate** DNA. For realization of toxic effect catalytic subunit A of RIP II type should by delivered to cell by B subunit. Without B subunit A chain is not toxic but can be used for blood plasma DNA's inactivation due to its polynucleotide-adenylglicozidase activity.

Group 6 - 6 mice transplanted with low metastatic LLC strain + two intravenous injections of total DNA fraction from mice transplanted with highly metastatic strain on the 7th and 8th day after transplantation(0,05 mkg of DNA was dissolved in 500 mkl of fresh heparinized blood before injection. Total DNA fraction was enzymatically methylated before administration (I.Muiznieks et.al.,FEBS Letters,1994,v.344,pp.251-254).

Number of metastaic nodules in lungs was estimated on the 15th day after transplantation.

Results of the experiments are presented in the table.

Group	Ncp.
1	12,0
2	22,5
3	14,1
4	15,5
5	15,1
6	12,3

Received data indicates that all used methods inhibited ability of blood plasma DNA of mice with highly metastatic LLC tumor strain to increase metastasizing process of low metastatic LLC tumor strain.

Example 18

Influence of DNase I therapy on the development of somatic mosaicism.

Frequency of HPRT gene's mutations in blood T-lymphocytes was studied as a model of somatic mosaicism. Human HPRT gene (chromosome Xq26) encodes enzyme that is constitutionally expressing but not essential and is involved in metabolism of purine nucleotides. Cloning was done according to Bigbee W. method (Bigbee W. Et al., Mutation Res.,1998,v.397,pp.119-136).

Lymphocytes from peripheral blood of 8 patients subjected to 3 week course of immunostimulating therapy by Neovir after surgical elimination of tumor were used for cloning. 4 patients out from 8 were additionally subjected to intravenous administration of human recombinant DNase I (200 mkg/kg dose 4 times a day during three weeks).

Incidence of HPRT – deficient clones in the blood of patients that received DNase I therapy was 3 times less than in blood of patients that received only immunostimulating therapy.

Example 19

Influence of DNase I therapy on old rats' life time.

White not thoroughbred 24 month old rats were used in experiment. In the study group (10 animals) rats starting from the age of 24 months were subjected to intravenous administration of polysialated human DNase I at 500 mg/kg (equivalent dose of non-modified enzyme) 2 times a week during 2 months. Control group of animals got the administration of placebo. Average life time of rats from control group was 27,8 months. Average life time of rats from experimental group was 30,1 months.

Example 20

Influence of DNase I therapy on viability of pancreatic β̃ cells and endothelium of aorta.

β̄ cells of human embryonic pancreas and endothelial cells of
5   human aorta were used for primary cell culture formation. DNA isolated from plasma of patient with severe II-type diabetes complicated by systemic atherosclerosis (0,0025 mkg per 1 ml of culture media) was added to cell culture 24 hours after passage.

In other experimental series DNA of the patient before
10   introduction to cell culture was processed with enzymatic methylation. Amount of viable cells was estimated 24 hours later with trypan blue staining.

Results of experiment are presented in the table.

Percentage of viable cells 48 hours after subculturing.

Cells	Control	Patient's DNA	Methylated DNA
B-cells	73%	43%	61%
Endothelium	62%	35%	55%

15

Industrial applicability

Current invention justify that destruction (binding, inactivation) of circulating blood plasma DNA in course of oncological and bacterial
20   diseases possess significant therapeutic effect.

Invention is confirmed by the fact that blood plasma DNA of patients contains unique genes that takes part in formation and maintenance of tumor's "malignant phenotype".

Binding, destruction or inactivation of DNA circulating in blood plasma possess therapeutic effect at diseases which development is connected with accumulation and spreading of somatic mutations in host's cells.

5 Blood plasma circulating DNA can be destructed, inactivated or eliminated from blood plasma by DNases, sorbents, antibodies and other methods which provide inactivation by destroying, binding or modification of circulating DNA.

Treatment directed to the destruction of blood plasma DNA lead

10 to significant atitumor effect while its own toxicity is immaterial.

Treatment directed to the destruction of blood plasma DNA if combined with traditional antitrumor therapy lead to significant antitumor effect.

Efficiency of the treatment directed to blood plasma DNA's

15 destruction can be estimated by monitoring of DNA amount and amount of tumor's genetic markers in blood plasma of patient who receive such therapy.

Cloning and sequencing of blood plasma DNA from oncological patient's can be used for studying of genetic processes that take part in

20 oncogenesis and identification of new genes connected with oncogenesis processes.

Cloning and sequencing of healthy people's blood plasma DNA can be used for studying of genome's operating in healthy individual and in the development of somatic diseases and in identification of new

25 genes that are involved in these processes.

Pharmaceutical compositions containing components that inactivate by destroying, binding and modification the blood plasma DNA could be used for treatment of patients with oncological diseases, infections, somatic diseases and for prolongation of life time.

5   For providing effective therapeutic exposition of active component that is necessary for blood plasma DNA' destruction, and for therapeutic effect achievement pharmaceutically acceptable compositions and modifications, drug delivery systems are used providing maximal systemic circulations of the active component in

10   blood plasma and its contact with DNA circulating in plasma. The main way of administration is intravenous. But other ways of administration that provide coming of active component in to systemic circulation, such as subcutaneous, intramuscular, inhalation, intraperitoneal and others can also be used. Doses and regimes of administration are determined by

15   nature of used active ingredient and way of administration. The effect is controlled by the level of DNA's contents and its dynamics in blood plasma, presence of tumor, infectious and other genetic markers, and appearing of positive clinical dynamics of disease.

20